International Rules for Seed Testing 2022

Validated Seed Health Testing Methods

7-001b: Detection of *Alternaria dauci* in *Daucus carota* (carrot) seed by malt agar method

Including changes and editorial corrections adopted at the online Ordinary General Meeting 2021

Effective from 1 January 2022

Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

Disclaimer

Whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description, ISTA shall not be liable for any loss or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

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7-001b: Detection of *Alternaria dauci* in *Daucus carota* (carrot) seed by malt agar method

Host: *Daucus carota* L.
Pathogen(s): *Alternaria dauci* (J.G.Kühn) J.J.Groves & Skolko, syn. *A. porri* f.sp. *dauci* (J.G.Kühn) Neerg., syn. *A. carotae* (Ellis & Langlois) Stevenson & Wellman

Prepared by: International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg)

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Revision history

Version 1.0, 2003-01-01
Version 1.1, 2013-01-01: Definition of sample size
Version 1.2, 2014-01-01: Addition of positive control; addition of streptomycin sulphate; common name of host added
Version 1.3, 2016-01-01: New figures 1a, 1b
Version 1.4, 2017-01-01: Reporting results revised
Version 1.5, 2021-01-01: Sample size added and Methods revised

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 4 and revised in 1987 (Gambogi, 1987). It has been slightly modified following studies conducted by the International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg) in 1999 and 2001 (Van Bilsen. 2003). The studies compared blotter and malt agar methods and concluded that the two were equivalent. The major modification is evaluation after 10 d incubation rather than 7 d. Note that seeds can be simultaneously tested for the presence of *Alternaria radicina* using the same method (see method 7-002b).

Treated seed

Seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Sample size

The sample (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Malt agar plates with streptomycin sulphate: 90 mm Petri dishes, one plate per ten seeds
Incubator: Operating at 20 ±2 °C, equipped with timer-controlled near-ultraviolet lights (NUV, peak at 360 nm, e.g. colour number 08, Philips; BLB Sylvania).

Sample preparation

It is vital to exclude any possibility of cross-contamination between seed samples. This can be achieved by swabbing/spraying equipment and gloved hands with 70 % ethanol.

Methods

Critical control points are indicated by CCP.

1. Pretreatment: None.
2. Plating
2.1 Aseptically place a maximum of 10 seeds evenly spaced on the agar surface of each malt agar plate.
2.2 Positive control (reference material): Aseptically place seeds evenly spaced (CCP), onto the agar surface of an appropriate number of malt agar plates to obtain the reference culture, or plate a reference culture on one malt agar plate. The number of plates required will depend on the level of contamination of the positive control seed lot.
3. Incubate plates for 10 d at 20 ±2 °C, with alternating 12 h periods of darkness and NUV light. Plates should be approx. 25 cm below the lights and should not be stacked.

4. Subculture a reference culture to a malt agar plate at the same time seeds are plated and incubate with the test plates.
5. Examine plates visually, and under a stereoscopic microscope at ×30 magnification, for fungal growth. Use a magnification of ×50 to ×80 for identification of conidia. Colonies of *Alternaria dauci* are brown or dark brown with olive-grey aerial mycelium and produce a brown diffusible pigment in the medium. Conidiophores are simple or slightly branched (Fig. 1), arising singly or in small groups from the surface of the seed or on aerial mycelium. Conidia are usually solitary, obclavate, up to 450 µm long (including beak), pale olivaceous brown at first, becoming brown with age, with a long pale beak up to 3 times the length of the body (Ellis, 1971). Groups of sunken conidia are sometimes visible by the emerging clusters of their bright long beaks (Fig. 1c). Compare with positive control. Record the number of infected seeds in each plate **(**CCP**)**

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

Reporting results: The result of a seed health test should indicate the scientific name of the pathogen detected and the test method used. When reported on an ISTA Certificate, results are entered under 'Other Determinations'.
The report must indicate the number of seeds tested.
In the case of a negative result (pathogen not detected), the results must be reported as 'not detected'.
In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Specific training

This test should only be performed by persons who have been trained in fungal identification or under their direct supervision.

Critical control points (CCP)

- Contaminants may greatly compete with the pathogen on the non-selective medium making detection laborious and difficult (Step 5).
- The malt agar source can influence the results. Whenever a new batch of malt agar is used a check on the quality should be made using a reference lot with a known infection level (Preparation of malt agar).

Media and solutions

Malt agar + streptomycin

Malt agar (CCP): as specified by manufacturer
Streptomycin sulphate: 50 mg
Distilled/deionised water: 1000 ml

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Steam to dissolve.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow agar to cool to approx. 50 °C and add streptomycin sulphate dissolved in water.
6. Pour 15–22 ml of molten agar into 90 mm Petri plates and allow to solidify at room temperature (20–25 °C) for 24 h before use.

Storage

Prepared plates may be stored at room temperature or at 4 °C for up to one month before use.

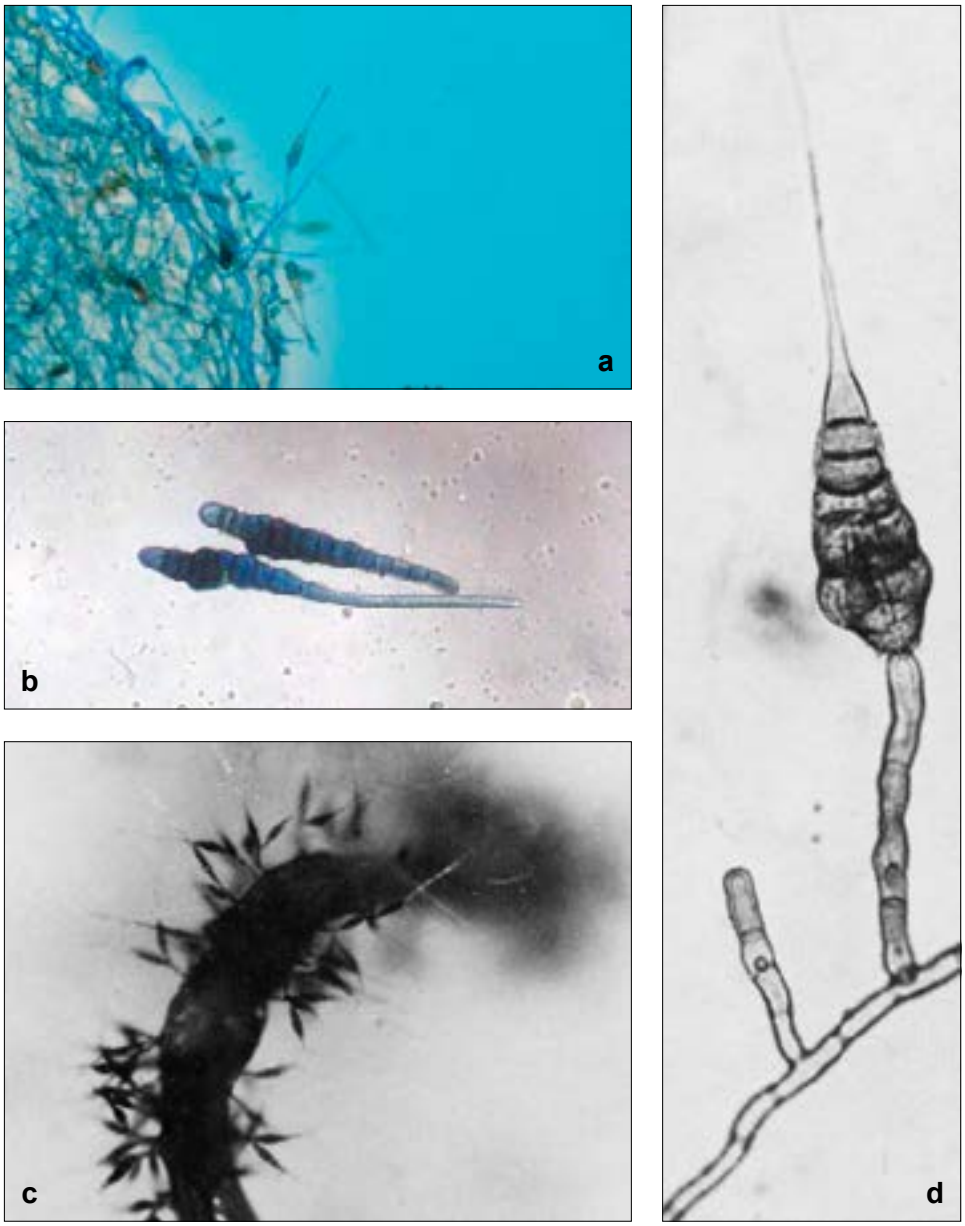

Figure 1. a Conidia of *Alternaria dauci* on simple or slightly branched conidiophores. **b** Single conidia. **c** Conidia of *A. dauci* on simple or slightly branched conidiophores borne on a single rootlet initial. **×**80. **d** Conidium and simple conidiophores. **×**350.

References

Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 609 pp.

Gambogi, P. (1987). *International Seed Testing Association, Handbook of Seed Health Testing, Working Sheet No. 4 (2nd Ed.)*: Alternaria dauci *on* Daucus carota. International Seed Testing Association, Zurich, Switzerland.

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Roberts, S. J., Phelps, K., Taylor, J. D. & Ridout, M. S. (1993). Design and Interpretation of seed health assays. In *Proceedings of the First ISTA Plant Disease Committee Symposium on Seed Health Testing* (ed. J. W. Sheppard), pp. 115–125, Agriculture Canada, Ottawa, Canada.

Validation references

ISTA (2003). Report of a comparative test on *Alternaria dauci* and *Alternaria radicina* on carrot seed. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.